JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2005, p. 476–478 Vol. 43, No. 1
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.1.476–478.2005

Activities of Available and Investigational Antifungal Agents against *Rhodotorula* Species

D. J. Diekema,[1,2]* B. Petroelje,[1] S. A. Messer,[2] R. J. Hollis,[2] and M. A Pfaller[2,3]

Departments of Internal Medicine[1] and Pathology,[2] Roy J. and Lucille A. Carver College of Medicine, and Department of Epidemiology,[3] College of Public Health, University of Iowa, Iowa City, Iowa

Received 6 July 2004/Returned for modification 28 August 2004/Accepted 13 September 2004

Rhodotorula **species are emerging pathogens in immunocompromised patients. We report the in vitro activities of eight antifungals against 64 *Rhodotorula* isolates collected in surveillance programs between 1987 and 2003. *Rhodotorula* strains are resistant in vitro to fluconazole (MIC at which 50% of the isolates tested are inhibited [MIC_{50}], >128 μg/ml) and caspofungin (MIC_{50}, >8 μg/ml). Amphotericin B (MIC_{50},1 μg/ml) and flucytosine (MIC_{50}, 0.12 μg/ml) are both active in vitro, and the new and investigational triazoles all have some in vitro activity, with ravuconazole being the most active (MIC_{50}, 0.25 μg/ml).**

Rhodotorula species, yeasts that belong to the family *Cryptococcaceae*, have been increasingly recognized as important human pathogens (1, 4, 7, 8, 10–12, 14, 18, 21, 23). Immunocompromised patients, particularly those with central venous catheters or other indwelling devices, are at highest risk for infection (10, 14, 18). While *Rhodotorula* strains appear to be less virulent than the more common yeast pathogens such as *Candida* and *Cryptococcus neoformans*, *Rhodotorula* infection has been associated with a crude mortality of up to 15% (12) and can cause sepsis syndrome and other life-threatening complications (4, 10, 13). *Rhodotorula* bloodstream infections have been successfully managed with line removal alone, antifungal therapy without line removal, and with a combination of these approaches (7, 10). Regarding choice of antifungal therapy, previously reported data have shown amphotericin B and flucytosine (5-FC) to have good in vitro activities and fluconazole and the echinocandins to have poor in vitro activities (2, 5, 6, 20, 23). However, most reports describe fewer than 10 organisms, not all utilize standard NCCLS methodology, and only a few report data on the newer extended-spectrum triazoles (2, 5, 6, 23).

For these reasons, we decided to examine the in vitro activities of agents against over 60 *Rhodotorula* isolates we have collected as part of antifungal resistance surveillance surveys since the late 1980s, utilizing standardized NCCLS methods and including new and investigational antifungal agents. To our knowledge, this represents the largest collection of clinical *Rhodotorula* isolates for which susceptibility test results obtained by standard NCCLS methods have been reported.

Sixty-four isolates of *Rhodotorula* were collected between 1987 and 2003 as part of several antifungal surveillance surveys (19). Sixty (94%) were human clinical isolates, the majority (n = 36 [56%]) from bloodstream (n = 28) or other sterile sites (n = 8). All isolates were stored as suspensions in sterile distilled water at room temperature until used in the study. Prior to testing, each isolate was subcultured at least twice on potato dextrose agar plates (Remel, Lenexa, Kans.) to ensure purity and optimal growth. We confirmed species identification by using the Vitek and API yeast identification systems (bioMerieux, Inc., Hazelwood, Mo.) supplemented by conventional methods as needed (9). Voriconazole (Pfizer), fluconazole (Pfizer), itraconazole (Janssen), ravuconazole (Bristol-Myers Squibb), posaconazole (Schering), and caspofungin (Merck) were obtained from their respective manufacturers, and 5-FC and amphotericin B were obtained from Sigma (St. Louis, Mo.). Stock solutions were prepared in dimethyl sulfoxide, polyethylene glycol (itraconazole), or water (5-FC) and further diluted in RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma) and were dispensed into 96-well microdilution trays. Trays containing a 0.1-ml aliquot of the appropriate drug solution (two times the final concentration) in each well were subjected to quality control (QC) testing and then sealed and stored at −70°C until used in the study. Fluconazole and 5-FC concentrations in the wells ranged from 0.12 to 128 μg/ml and 0.06 to 64 μg/ml, respectively, while concentrations of all other agents ranged from 0.007 to 8 μg/ml.

Susceptibility testing was performed by the broth microdilution method according to the recommendations of NCCLS document M27-A2 (16). An inoculum suspension to match the turbidity of a 0.5 McFarland standard, diluted to a concentration of 1.0×10^3 to 5.0×10^3 cells per ml, was standardized spectrophotometrically, and an aliquot of 0.1 ml was added to each well of the microdilution tray (final inoculum, 0.5×10^3 to 2.5×10^3 cells/ml). In each case, the inoculum size was verified by colony counting. The microdilution trays were incubated at 35°C. However, two isolates were also incubated at 30°C because growth at 35°C was insufficient for endpoint determination. The MIC endpoints were read visually following 72 h of incubation. The MIC of amphotericin B was defined as the lowest concentration that produced complete inhibition of growth (first clear well). The MICs of all other tested agents were defined as the lowest concentration that produced a prominent decrease in turbidity compared to the drug-free control well.

* Corresponding author. Mailing address: Medical Microbiology Division, C606 GH, Department of Pathology, University of Iowa College of Medicine, Iowa City, IA 52242. Phone: (319) 356-8615. Fax: (319) 356-4916. E-mail: daniel-diekema@uiowa.edu.

TABLE 1. MICs of eight antifungal agents against 64 *Rhodotorula* isolates determined by reference broth microdilution method

Species (no. of isolates tested)	Antifungal agent	No. of isolates susceptible at MIC (μg/ml) of:										
		≤0.06	0.12	0.25	0.5	1	2	4	8	16[a]	32	≥64
R.glutinis (29)	Amphotericin B				1	28[b,c]					—[d]	—
	Ampho B Etest		2	2	17[b]	6[c]	2					
	5-FC	13	10[b]	6[c]								
	Caspofungin									29[b,c]	—	—
	Fluconazole											29[b,c]
	Itraconazole					8	9[b]	2	3	7[c]	—	—
	Posaconazole					8	17[b]	4[c]			—	—
	Ravuconazole	3	9	7[b]	8[c]	2					—	—
	Voriconazole					10	12[b]	7[c]			—	—
R. mucilaginosa (24)	Amphotericin B					23[b,c]	1				—	—
	Ampho B Etest			7	9[b]	7[c]	1					
	5-FC	6	7[b]	10[c]	1							
	Caspofungin									24[b,c]	—	—
	Fluconazole											24[b,c]
	Itraconazole				1	10	4[b]	1	2	6[c]	—	—
	Posaconazole				1	3	13[b]	2	1	4[c]	—	—
	Ravuconazole	3	6	6[b]	2	5[c]	2				—	—
	Voriconazole			1	2	6	6[b]	6	2[c]	1	—	—
All *Rhodotorula* (64)	Amphotericin B				3	60[b,c]	1				—	—
	Ampho B Etest		4	11	26[b]	14	4[c]	1	1			2
	5-FC	25	20[b]	17[c]	2							
	Caspofungin								4	60[b,c]	—	—
	Fluconazole										1	63[b,c]
	Itraconazole				3	22	18[b]	3	5	13[c]	—	—
	Posaconazole				2	14	37[b]	6[c]	1	4	—	—
	Ravuconazole	6	17	15[b]	13	8[c]	5				—	—
	Voriconazole			1	2	18	23[b]	17[c]	2	1	—	—

[a] For all agents except 5-FC and fluconazole, numbers in this column represent all isolates for which the MIC was >8 μg/ml.
[b] MIC_{50}.
[c] MIC_{90}.
[d] —, dilutions not included in testing for this antifungal agent.

Because broth dilution testing of amphotericin B may not be sensitive enough to detect some antifungal-resistant yeasts (e.g., *Candida*) (22), we also tested this agent by the Etest method as we have previously described for testing amphotericin B against *C. neoformans* (15).

QC testing was performed in accordance with NCCLS document M27-A2 and limits established by Barry et al. (3), using *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 (16).

Of the 64 *Rhodotorula* isolates examined, 29 were *R. glutinis*, 24 were *R. mucilaginosa*, 5 were *R. minuta*, and 6 were not identified to the species level. The results of susceptibility testing overall and by the two major species are outlined in Table 1.

Amphotericin B MICs by broth dilution clustered around a value of 1 μg/ml, but Etest demonstrated a wider range of MICs and detected eight isolates for which the MIC was >1 μg/ml, raising the possibility that some *Rhodotorula* isolates may be less susceptible in vitro or in vivo than prior data would suggest.

5-FC was the most active in vitro of all the agents tested, with all isolates inhibited at ≤0.5 μg/ml. This agent has been used in treatment of serious *Rhodotorula* infections (4, 17), and it should be considered a first-line agent in combination with amphotericin B for invasive *Rhodotorula* infections.

Our data confirm that the echinocandin caspofungin does not have in vitro activity against *Rhodotorula*, as would be expected given the lack of activity of echinocandins against other members of the family *Cryptococcaceae* (6).

Our collection of *Rhodotorula* isolates is large enough to highlight the differences in potency within the azole antifungal class. As has been previously described, fluconazole is not active in vitro, and the MIC of itraconazole for almost 30% of isolates is >4 μg/ml. The newer broad-spectrum azoles are more active, with MICs at which 90% of the isolates tested are inhibited (MIC_{90}s) of ≤4 μg/ml. Interestingly, of the broad-spectrum azoles tested, ravuconazole MICs were about four-fold lower than voriconazole or posaconazole MICs (MIC_{50}/MIC_{90}, 0.25/1 versus 2/4).

We noted no significant differences in the activities of any of the tested agents according to the species of *Rhodotorula*.

In summary, our results confirm previous reports of the in vitro activities of amphotericin B and 5-FC against *Rhodotorula*, as well as the absence of activity of the commonly used agents fluconazole and caspofungin. Each of the newer triazoles has some activity and may be useful as an alternative agent, but additional clinical experience is needed. Ravuconazole in particular showed excellent in vitro activity, and if this agent were further developed for clinical use, it might have a role in treatment of life-threatening or refractory *Rhodotorula* infections.

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